Environmental Health Perspectives
Vol. 86, pp. 323–326, 1990

Cytogenetic Studies in Human Populations Exposed to Gas Leak at Bhopal, India

by Bani Bandana Ghosh,* Susmita Sengupta,* Ajoy Roy,* Srabani Maity,* Sujita Ghosh,* Geeta Talukder,* and Archana Sharma*

Frequencies of chromosomal abnormalities, sister chromatid exchanges, and replicative index were assessed following peripheral lymphocyte culture in 129 individuals from Bhopal, India. Of these, 83 persons (40 male and 43 female) had been exposed directly to the methyl isocyanate (MIC) gas after the accident at the Union Carbide plant on December 2 and 3, 1984. The remaining 46 samples were taken from age-matched unexposed persons in the same city. Chromosome aberrations were recorded at first cycle metaphase (M_1) and sister chromatid exchanges, at second cycle metaphase (M_2), following standard schedule. The frequency of chromosomal aberrations was, in general, higher in individuals from the exposed populations, with the females showing a higher incidence. Nondisjunction of chromosomes or laggard was rare. The frequencies of sister chromatid exchanges and depression in mitotic and replicative indices could not be related to exposure or sex. The persistence of chromosomal abnormalities in the form of replicating minutes and exchange configurations, even 1114 days after exposure to the gas, may indicate a residual effect on T-cell precursors.

Introduction

The disaster in the Union Carbide plant at Bhopal, India, between December 2 midnight and the early morning of December 3, 1984, affected more than 14,000 individuals (*1*). The inhabitants in the township of Bhopal were exposed in different degrees, depending on their proximity to the plant and atmospheric factors. A major lacuna in treating the exposed persons was the lack of adequate information on the toxicological effects of MIC (*2*). The unprecedented mortality and morbidity rates initiated detailed studies in experimental animals (*3*) and in some exposed individuals (*4*). The resultant publications show the wide spectrum covered by the toxic effects of MIC (*1–4*). Individual publications are now available on the chemistry of the reaction (*5*) as well as its genotoxic and clastogenic effects on laboratory test systems, ranging from Salmonella, Drosophila, and mice *in vivo* to Chinese hamster ovary cells *in vitro* (*6–8,11,12*). Sister chromatid exchanges (SCEs) have been reported in some exposed persons after the accident (*4,9,10,13*).

*Human Genetics Unit, Center for Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, 35 Ballygunj Circular Road, Calcutta 700019, India.

Address reprint requests to B. Ghosh, Human Genetics Unit, Center for Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, 35 Ballygunj Circular Road, Calcutta 700019, India.

In the present case, chromosomal analysis was carried out in cases randomly selected in the exposed area up to 1114 days after exposure to the gas in order to study the residual effects, if any, on the chromosomes.

Material and Methods

The 129 individuals studied were categorized into those persons directly exposed with history of exposure and those who were not exposed, that is, residents in neighboring areas where the gas did not spread. The group of exposed persons was chosen through a random computer-based selection of listed survivors. The control group was taken from the unexposed population and was matched by age and sex.

Peripheral venous blood was collected in heparinized vials and plasma was separated out by gravity sedimentation. After an hour, leukocytes were inoculated to RPMI-1640 medium (GIBCO, Grand Island, NY), supplemented with 20% heat-inactivated fetal calf serum (Sera Lab., England) and phytohemagglutinin (M Form, GIBCO) at 0.2 mL/5 mL of culture.

In the set for the study of sister chromatid exchanges, 6.0 μg of 5-bromo-2-deoxyuridine (BrdU, Sigma Chemical Co., St. Louis, MO) was added per 1.0 mL of the culture medium in the dark to prevent photoinactivation.

Four cultures were maintained for each subject in two

replicate sets in the dark at 37°C. The period of incubation was 48 hr for the study of chromosome aberrations and 72 hr for SCEs; 2 hr before termination of the culture, colchicine (40 μg/mL) was added to each cul-After further incubation for 2 hr, cells were centrifuged into pellets and treated successively in hypotonic solution (0.09% NaCl in deionized water) and fixative (methanol acetic acid 3:1) for 25 to 30 min at 37°C. The cells were centrifuged and resuspended in fixative three times. Air-dried slides were prepared and stained in Giemsa according to the standard procedure (*14–16*) with slight modifications as required.

The slides were coded and scored blind by two observers. The parameters recorded in each subject were (*17–22*): *a*) chromosomal abnormalities (including damaged cells; total aberrations with and without gaps, breaks per damaged cell) recorded in 100 first-cycle scattered metaphases (M_1) per subject; *b*) sister chromatid exchanges in 50 scattered complete second-cycle metaphases (M_2) per subject; and *c*) cell cycle kinetics in 200 metaphases per subject. Parameters included replicative index (RI) and cell cycle metaphases in first (M_1), second (M_2), and third (M_3) cycles (*23*). The aberrations for the first three sets of parameters were compared by Student's *t* test between exposed and unexposed populations of both sexes.

Results and Discussion

Chromosomal aberrations were found to occur in statistically higher frequencies in the exposed group as compared to the control, especially in female subjects (Table 1). The types of abnormalities recorded were chromosome breaks, gaps, dicentrics, rings, and triradial and quadriradial configurations. Persistent, replicating minute chromosomes and quadriradial configurations were seen even after 1114 days (Figs. 1–4). In general, the number of breaks per cell was higher in exposed females as compared to unexposed ones. In males the difference was not significant. These observations are contradictory to the record of the higher incidence of micronuclei in male mice subjected to MIC *in vivo* (*7,8*).

Table 1. Effect of methyl isocyanate—chromosomal aberration related to sex.

Sex	Exposed type	Number of subjects	Cells scored	Total damaged cells[a]	Breaks/cell[a] + Gaps	Breaks/cell[a] − Gaps	Aberration/damaged cell[a]
Male	Exposed	39	4141	5.77 ± 1.99	0.072 ± 0.024	0.064 ± 0.023	1.27 ± 0.32
	Unexposed	20	1884	5.49 ± 1.83	0.068 ± 0.031	0.056 ± 0.025	1.22 ± 0.24
Female	Exposed	43	3963	6.74 ± 2.42	0.094 ± 0.027	0.077 ± 0.025	1.44 ± 0.37
	Unexposed	27	2336	5.46 ± 2.16*	0.063 ± 0.032‡	0.050 ± 0.021‡	1.15 ± 0.36†
Male	Exposed	39	4141	5.77 ± 1.99	0.072 ± 0.024	0.064 ± 0.023	1.27 ± 0.32
Female		43	3963	6.74 ± 2.42	0.094 ± 0.027‡	0.077 ± 0.025*	1.44 ± 0.37*
Male	Unexposed	20	1884	5.49 ± 1.83	0.068 ± 0.031	0.056 ± 0.025	1.22 ± 0.24
Female		27	2336	5.46 ± 2.16	0.063 ± 0.032	0.050 ± 0.021	1.15 ± 0.36

[a]Figures are expressed as mean ± SD significant in Student's *t* test.
*$p<0.05$.
†$p<0.01$.
‡$p<0.001$.

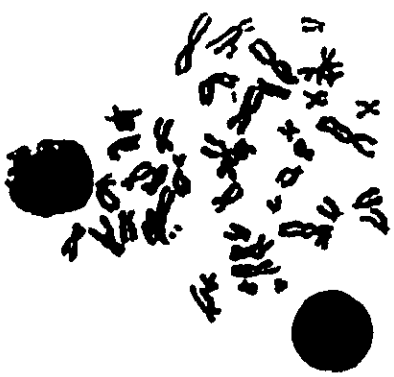

FIGURE 1. Replicating minutes.

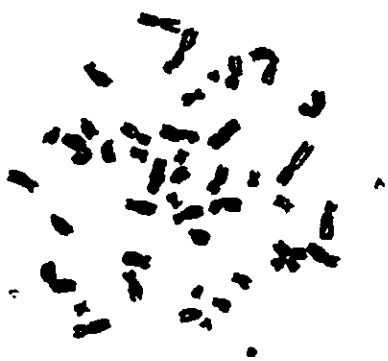

FIGURE 2. Quadriradial and dicentric configurations.

FIGURE 3. Endoreduplication.

FIGURE 4. Sister chromatid exchange.

Table 2. Effect of methyl isocyanate—sister chromatid exchanges (SCEs) related to sex.

Sex	Exposure type	Number of subjects	Total cells scored	Total SCEs counted	Range of Mean SCEs	SCEs/cell Mean ± SD
Male	Exposed	40	1537	15722	5.40 – 19.17	10.69 ± 3.41
	Unexposed	19	925	10770	6.46 – 17.67	11.61 ± 1.92
Female	Exposed	42	1473	15307	5.94 – 19.82	10.87 ± 3.49
	Unexposed	26	1159	13796	6.60 – 23.39	12.54 ± 3.57
Male	Exposed	40	1537	15722	5.40 – 19.17	10.69 ± 3.41
Female		42	1474	15307	5.94 – 19.82	10.87 ± 3.49
Male	Unexposed	19	925	10770	6.46 – 17.67	11.61 ± 1.92
Female		26	1159	13796	6.67 – 23.39	12.54 ± 3.57

The frequencies of SCEs did not differ markedly between exposed and unexposed populations. In general, the range was above the baseline ranges of 4 to 14 per cell reported earlier (*24*). The range of SCEs, number of breaks per cell, and percentage of cells with SCE could not be related to exposure or the sex of the person (Table 2). These observations do not support an earlier isolated report of increased frequency of SCEs in individuals exposed to MIC during the Bhopal accident (*10*). In another communication, no relation has been observed to factors such as smoking, alcohol intake, and pregnancy (*13*). The replicative index was not altered significantly between the exposed and unexposed populations.

The study indicates an apparent increase in the frequency of chromosomal aberrations in exposed females 1114 days after exposure to the gas. Since the selection was made as double blind in order to facilitate independent checks by two observers, the history and clinical features (if any) of these cases have not yet been fully related to the observations. The incidence of chromosomal alterations like dicentrics, rings, and quadriradial configurations even after 1114 days may indicate persistent clastogenic effects. Because the lesions induced by chemicals are mostly S dependent for expression in the subsequent divisional cycle, the damaged T-lymphocytes may remain circulating for long periods, and these aberrations can be observed only if the cells are stimulated to divide *in vitro* (*25*). The results are comparable to those obtained following exposure to *p*-dioxane (*26*).

Some of the females with persistent chromosomal aberrations have a history of fetal loss, which may have been the consequence of exposure to MIC. None of them has been exposed to any other known clastogenic agent except a single chest X-ray. The majority of the exposed females studied were housewives and the males were day laborers.

At present the observations are not complete and more needs to be done. In assessing the effects of exposure to toxic chemicals such as MIC, the sex of the subject and the physiological and nutritional status as well as other compounding factors such as genetic composition have to be taken into account (*27,28*). The persistence of aberrations even after 1114 days indicates the possibility of a higher susceptibility to chromosome damage of persons exposed to MIC.

REFERENCES

1. Government of India. Report on the Factors Related to Bhopal Toxic Gas Leakage. Government of India, December, 1985.
2. Kimmerle, G. and Eben, A. Toxicity of methyl isocyanate and how to determine the quantity of air. Arch. Toxicol. 20: 235–241 (1964).
3. National Toxicology Program. The toxicity of methyl isocyanate. Environ. Health Perspect. 72: 1–200 (1986).
4. Bhopal gas tragedy. Bhopal gas disaster: clinical and experimental studies. Ind. J. Exp. Biol. 26: 204 (1988).

5. Worthy, W. Methyl isocyanate: the chemistry of a hazard. Chem. Eng. News 63: 27–33 (1985).
6. Mason, J. M., Zieger, E., Hayworth, S., Ivett, J., and Valencia, R. Genotoxicity studies of methyl isocyanate in *Salmonella*, *Drosophila* and cultured Chinese hamster ovary cells. Environ. Mutagen. 9: 19–28 (1987).
7. Kligerman, A. D., Campbell, J. A., Erexson, G. A., Allen, J. W., and Shelby, M. D. SCE in lung and peripheral blood lymphocytes of mice exposed to MIC by inhalation. Environ. Mutagen. 9: 29–36 (1987).
8. Tice, R. R., Luke, C. A., and Shelby, M. D. Methyl isocyanate: an evaluation of *in vivo* cytogenetic activity. Environ. Mutagen. 9: 37–58 (1987).
9. Goswami, H. K., Guron, C., Jain, N., and Goswami, I. Genetic assessment of Union Carbide gas tragedy in Bhopal. I. Effects on somatic chromosomes and haemoglobin. Bionature 4: 88–90 (1984).
10. Goswami, H. K. Cytogenetic effects of MIC exposure in Bhopal (India). Hum. Genet. 74(1): 81–84 (1986).
11. Saxena, A. K., Singh, K. P., Nagle, S. L., Gupta, B. N., Ray, P. K., and Srivastav, R. K. Effect of exposure to toxic gas on the population of Bhopal. Part IV: Immunological and chromosomal studies. Ind. J. Exp. Biol. 26(3): 173–176 (1988).
12. Dutta, K. K., Gupta, G. S. D., Mishra, A., Joshi, A., Tandon, G. S.,, and Ray, P. K. Inhalation toxicity studies of methyl isocyanate (MIC) in rats. Part I: Pulmonary pathology and genotoxicity evaluation. Ind. J. Exp. Biol. 26(3): 177–182 (1988).
13. Talukder, G., and Sharma, A. The Bhopal accident: its after effects. In: Management of Hazardous Materials and Wastes (S. K. Majumdar, E. W. Miller, and R. F. Schmalz, Eds.), Pennsylvania Academy of Science Publication, Easton, PA, 1989, pp. 409–417.
14. Goto, K., Akematsa, T., Shimazu, H., and Sugiyama, T. Simple differential staining of sister chromatids after treatment with staining. Chromosoma 53: 223–230 (1975).
15. Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. N., and Hungerford, A. Chromosome preparation of leukocytes cultured from human peripheral blood. Exp. Cell Res. 20: 613–616 (1960).
16. Perry, P., and Wolff, S. New Giemsa method for the differential staining of sister chromatids. Nature 251: 156–158 (1974).
17. Brögger, A. The chromatid gap—a useful parameter in genotoxicology. Cytogenet. Cell Genet. 33: 14–19 (1982).
18. Brusick, D. J. Implications of treatment condition-induced genotoxicity for chemical screening and data interpretations. Mutat. Res. 189: 1–6 (1987).
19. De Serres, F. J., and Matsushima, T. Deployment of short-term assays for environmental mutagens and carcinogens. Mutat. Res. 182: 173–184 (1987).
20. International Atomic Energy Agency. Biological dosimetry: chromosomal aberration analysis for dose assessment. International Atomic Energy Agency, Technical Report No. 260, Vienna, Austria, 1986.
21. Preston, R. J., Sebastian, J. R. S., and McFee, A. F. The *in vitro* human lymphocyte assay for assessing the clastogenicity of chemical agents. Mutat. Res. 189: 175–183 (1987).
22. Scott, D. N., Dean, B. J., Kirkland, D., and Richardson, C. *In vitro* chromosome aberration assays. In: Report of the UK Environmental Mutagen Society Sub Committee on the Guidelines for Mutagenicity Testing (B. J. Dean, Ed.), 1983, p. 41–64.
23. Lamberti, L., Ponzelto, P. B., and Ardito, G. Cell kinetics and sister chromatic exchange frequency in human lymphocytes. Mutat. Res. 120: 192–199 (1983).
24. Ashby, J., and Richardson, C. R. Tabulation and assessment of 113 human surveillance cytogenic studies conducted between 1964 and 1984. Mutat. Res. 154: 111–133 (1985).
25. Carrano, A. V. and Natarajan, A. T. Considerations for population monitoring using cytogenetic techniques. Mutat. Res. 204: 379–406 (1988).
26. Berg, K. Genetic Damage in Man Caused by Environmental Agents. Academic Press, New York, 1979.
27. Scott, D., and Roberts, S. A. Extrapolation from *in vitro* tests to human risk: experience with sodium fluoride clastogenicity. Mutat. Res. 189: 47–58 (1987).
28. Sharma, A. Environment and genetic polymorphism in Indian populations. Plenary lecture at the meeting of American Association for the Advancement of Science, Chicago, February 15, 1987.